Temperature dependence of the short-range repulsion between hydrated phospholipid membranes: A computer simulation study

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The temperature dependence of the short-range water-mediated repulsive pressure between supported phospholipid membranes is calculated at two intermembrane separations using the grand canonical Monte Carlo technique. At both separations, the simulated pressure tends to decrease with temperature, in qualitative agreement with the experimental measurements by Simon and co-workers [Simon *et al.*, Biophys. J. **69**, 1473 (1995)]. The decrease in pressure originates, at least in part, from a slight dehydration of the membranes and the associated reduction in the hydration component of the pressure. © *2007 American Vacuum Society*. [DOI: 10.1116/1.2771541]

I. INTRODUCTION

The short-range repulsive pressure occurring between phospholipid membranes in water and aqueous solutions plays an important role in all biological phenomena in which the membranes come close together (e.g., in fusion).[1] This explains the substantial efforts undertaken in the last two decades to understand the nature of this repulsion. If the intermolecular interactions in the water-lipid system are treated in the two-body approximation, which is usually the case, the total water-mediated pressure p between two parallel lipid membranes can be broken up into a "direct" component, p_d, resulting from the direct interaction between the opposing membranes, and a hydration component, p_h, associated with the interaction of the membranes with the intervening water: $p = p_d + p_h$. The existing theoretical models for the short-range intermembrane repulsion can then be split into two groups depending on which pressure component is assumed to be dominant, p_h or p_d.

The models that treat the water-mediated repulsive pressure in terms of p_h ascribe the latter to dehydration of the membranes when they approach each other.[2] Inasmuch as the membrane surfaces are hydrophilic, the exclusion of water from the interfacial region leads to an energy loss, so that the resulting pressure is repulsive. On the molecular level, the origin of p_h is usually associated with orientational polarization or structuring of water near the membrane surface.[3]

The alternative approach implies the dominant role of p_d and treats the latter in terms of entropy-driven deviations of hydrated bilayer membranes from ideal planar geometry.[4] The most important deviations from planarity are membrane undulations and protrusions of individual lipid molecules and their headgroups into the aqueous phase. The undulation contribution to pressure becomes important at large intermembrane separations ($>10-15$ Å).[2] For supported (immobilized) membranes, as studied in surface force apparatus (SFA) measurements and in our simulations, membrane undulations are suppressed, so that p_d is determined by its protrusion component. As the opposing membranes come closer together, the thermal motion of protruded molecular fragments becomes more confined, which reduces their configurational entropy and results in a repulsive pressure. A detailed critical discussion of both protrusion and hydration models for the short-range intermembrane repulsion can be found elsewhere.[4–6]

An important argument to decide between the hydration and protrusion (entropic) models is provided by the temperature dependence of the intermembrane pressure. Theoretical treatments predict that the magnitude of protrusion pressure should increase with increasing temperature T.[4,6] By contrast, for hydration models the water-mediated pressure can be expected to be practically independent of T or to decrease with increasing T because of deterioration of the membrane-induced water structure.[4,6] Despite this contradiction, the temperature dependence of p has been invoked to support both the entropic and hydration models. Thus, Israelachvili and Wennerstroem[4] argued for the entropic model by referring to the experimentally observed increase in p on going from the gel to the fluid (liquid-crystalline) state of lipid bilayers with increasing temperature. Such an argument, however, does not seem to be quite convincing because the gel and fluid states are different phase states of bilayers, so that the increase in p can hardly be regarded as the sole effect of temperature. The gel-fluid transition and the associated increase in p can well be induced at a fixed temperature by increasing the hydration degree of the bilayers.[7]

A clearer understanding of the effect of temperature on the intermembrane pressure is provided by measurements of the p-T relation within a *single* lipid phase. Such measurements were reported by Simon *et al.*[8] who used the osmotic stress/x-ray diffraction method to compare the temperature dependence of pressure-distance curves in multi-bilayer suspensions of two phospholipids, one being in the gel state and the other in the fluid state. For the fluid phase lipid, where entropic pressures can be expected to be more significant than for the gel phase one, the short-range repulsive pressure plotted as a function of the inter-bilayer spacing D_w proved to be practically independent of temperature. Moreover, when plotted as a function of the lamellar repeat period D (which is, unlike D_w, a directly measured quantity), the in-

termembrane pressure showed, at a fixed D, a perceptible decrease with temperature. That is, the experiments by Simon et al.[8] supported the hydration model for the short-range repulsion.

In our recent papers,[9–11] we have attempted to dissect the intermembrane pressure into its physically distinct components in a straightforward way, via computer simulation. The simulations used the grand canonical Monte Carlo (GCMC) technique and were concerned with supported dilauroylphosphatidylethanolamine (DLPE) membranes. It was found that the short-range water-mediated repulsion was determined by p_h, whereas p_d remained attractive throughout the pressure range studied (0 to 5 kbar). The attractive p_d resulted mainly from a strong electrostatic attraction between the DLPE headgroups in the opposing membranes. No evidence was found in support of the protrusion model of the intermembrane repulsion.

In this paper, our previous GCMC simulations[9–11] are extended to an analysis of the temperature dependence of p to obtain further evidence on the role of hydration and entropic factors in the intermembrane repulsion.

II. METHOD

The force field and the simulation procedure used in our calculations were basically the same as described in detail in our previous papers.[9–11] In brief, the interactions between water molecules were described with the four-site TIP4P model.[12] No correction for the long-range electrostatic interactions was made because the original TIP4P model was calibrated without such a correction. The inter- and intramolecular energies of DLPE molecules were calculated with a united-atom AMBER-based force field developed by Smondyrev and Berkowitz.[13] The long-range part of the electrostatic interactions between DLPE molecules was evaluated in a group-based dipole-dipole approximation, while the shorter-range interactions were calculated as the direct sum of Coulombic potentials. The reliability of this calculation model was demonstrated in our previous article[9] by comparing the results for the electrostatic potential energy with the respective values obtained by the particle mesh Ewald (PME) technique.[14] The cutoff distances were 20 and 100 Å for the charge-charge and dipole-dipole interactions, respectively, which ensured an accuracy of 0.3% for the total electrostatic potential energy. Although such an accuracy is noticeably worse than that reported for PME, it hardly makes sense to strive for a better accuracy because the very representation of the continuous molecular charge distribution in terms of partial atomic charges introduces a much larger error in the electrostatic interaction energy.[15]

The mixed (water-lipid) interactions were treated using the geometric mean combination rule for the parameters of the Lennard-Jones potentials, whereas the electrostatic interactions were calculated with the same partial charges as used for the homomolecular (water-water and lipid-lipid) pairs. Here again, the long-range part of the electrostatic interaction energy was approximated by a sum of dipole-dipole potentials. The resulting water-lipid intermolecular energies
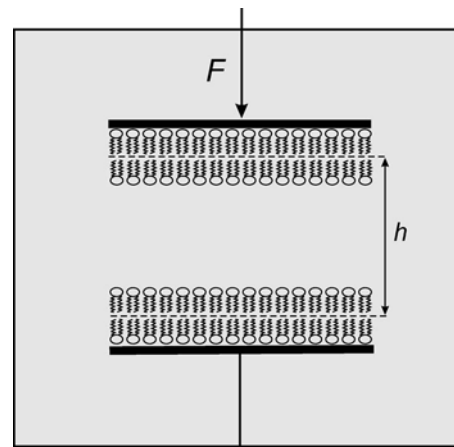

FIG. 1. Configuration of a SFA experiment with lipid membranes. The dashed lines show the position of generalized substrates when the outer monolayers are treated implicitly.

were damped by a scaling factor $\alpha = 0.93$. The need for damping the water-lipid interactions was justified in our previous study[11] based on a comparison of the calculated and experimental hydration degrees, n_w. The magnitude of α was adjusted so as to reproduce the experimental value of n_w at full hydration.

As noted in the Introduction, the hydrated membranes were simulated by the GCMC technique. We preferred GCMC over traditional molecular dynamics (MD) for two reasons. One is that GCMC, unlike MD, is well suited for simulating open confined systems, such as water confined between supported membranes in SFA experiments or water in multi-bilayer suspensions in osmotic stress measurements. In both cases, the confined water is in the chemical equilibrium and is allowed to exchange molecules with a bulk water reservoir. Similar conditions occur in nature, e.g., when two membranes approach each other prior to fusion. The other advantage of GCMC over MD is that it is more efficient in exploring the configurational space of the hydrated membrane. Unlike MD, GCMC is not tied to the time evolution of the system, and so it can efficiently explore the membrane's configurational space using various "unphysical" moves. In a GCMC simulation, the water molecule need not diffuse through the whole monolayer thickness to reach the middle of the membrane. The molecule can well do it by a particle insertion move.

The configuration of the model system was similar to that of a SFA experiment: Two DLPE bilayer membranes were supported on parallel solid substrates and brought to equilibrium with surrounding bulk water (Fig. 1). To reduce the computational cost of the simulations, the two outer monolayers, which were in direct contact with the solid substrates, were regarded as structureless generalized substrates for the adjacent inner monolayers, which were in contact with water. The interaction of the inner monolayers with their generalized substrates was treated in a mean-field manner using (10–4) inverse power atom-substrate potentials parameterized as described in Ref. 10.

The number of lipid molecules was fixed, i.e., the whole system was actually treated in a semi-grand canonical ensemble. To improve the acceptance of insertion and deletion attempts, we employed the excluded volume mapping[16] and Swendsen-Wang filtering[17] techniques, which allowed us to reject improbable positions for insertion or deletion of a water molecule based on a computationally inexpensive predictor. Further improvement in sampling efficiency was achieved due to implementation of a rotational bias procedure,[18] which made it possible to reject improbable molecular orientations.

The components of the water-mediated pressure were calculated through the grand canonical ensemble averages of the respective instantaneous forces. Thus, the hydration pressure was evaluated from

$$p_h = A^{-1} \left\langle \sum_{i,j} f_z^{i,j} \right\rangle_{\mu VT} - p_b, \tag{1}$$

where A is the membrane area, p_b is the bulk water pressure and $f_z^{i,j}$ is the z-component of the force experienced by the ith molecule in the upper membrane due to its interaction with the jth water molecule. To improve statistics, the pressure experienced by the lower membrane was also calculated and the results were averaged. The explicit expression for the atom-atom (Coulombic plus Lennard-Jones) contributions to $f_z^{i,j}$ can be found in our earlier article,[19] whereas the expression for dipole-dipole group-group contributions is readily derived by differentiation of the known formula for the dipole-dipole interaction energy.[15] The equation used in calculations of p_d is similar to Eq. (1), except that it does not contain p_b and the force refers to the interaction between lipid molecules i and j in different membranes.

III. RESULTS AND DISCUSSION

As in our previous simulations of hydrated DLPE membranes,[10,11] the simulation cell represented a rectangular prism with lateral dimensions $L_x = 35.8$ Å, $L_y = 45.8$ Å, corresponding to the area per molecule $A = 51.2$ Å^2, as observed experimentally for the fluid-phase DLPE at 308 K.[20] The height of the prism was determined by the separation between the generalized substrates, h. As shown earlier,[10,11] h is comparable with the repeat period D in multi-bilayer suspensions. (More precisely, the difference $D - h$ is estimated to be within $1 - 1.5$ Å.) The number of lipid molecules in each DLPE monolayer was 32.[21,22] The length of a typical GCMC run was 3×10^6 GCMC cycles, each comprising N_0 moves,
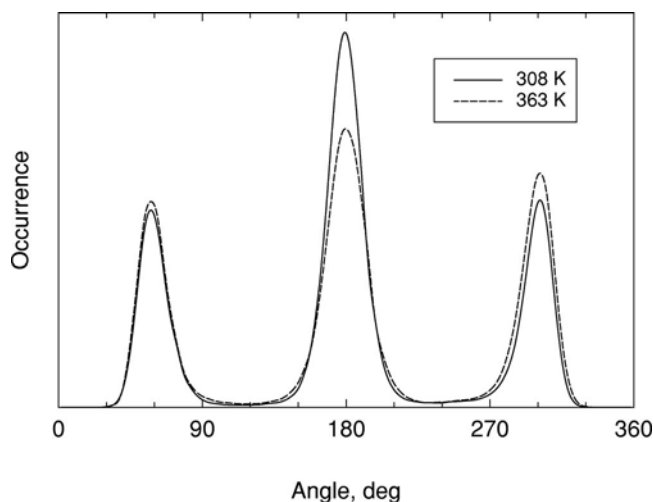

FIG. 2. Distribution of the dihedral angles determined by the last three C−C bonds of the γ-chains at $h = 38.6$ Å.

where N_0 is the initial number of water molecules in the given cycle. The total number of configurations attempted in a single run amounted to 1.2×10^9.

The GCMC simulations were performed at two selected substrate-to-substrate separations, $h = 38.6$ and 39.6 Å, and two temperatures, $T = 308$ and 363 K. The chemical potential of the confined system was specified by the "density-corrected" excess chemical potential, $\mu'' = \mu' + kT \ln d$,[19] where μ' is the excess (configurational) chemical potential[23] and d is the average water density in grams per cubic centimeter. The values of μ'' at the two above temperatures were determined in separate simulations of bulk water to give $\mu'' = -5.95$ and -5.34 kcal mole^{-1}, respectively. The areal density of the membranes was assumed to be independent of temperature. (Such a condition can, in principle, be reproduced in a SFA experiment, where the bilayer density can be adjusted to a desired value by varying the bilayer deposition parameters.)[24] To improve statistics, the ensemble averages for a given pair (h, T) were calculated in eight to ten independent GCMC runs differing in the starting configuration and/or the sequence of random numbers used in generating the run.

An analysis of the structural characteristics of the membranes did not reveal any significant and reproducible effect of temperature, except for the distribution of dihedral angles in the acyl chains, particularly in the chain ends. This is illustrated in Fig. 2, which presents the distribution of the

TABLE I. Simulated hydration degree, water-mediated pressure (kbar), and the components of the latter (Ref. 25). The probable errors are 0.1 for n_w and 0.2 kbar for the pressures.

	$h = 39.6$ Å				$h = 38.6$ Å			
T (K)	n_w	p	p_h	p_d	n_w	p	p_h	p_d
308	6.2	0.9	4.3	−3.4	6.0	2.7	5.3	−2.6
363	5.9	0.6	4.1	−3.5	5.6	2.1	4.9	−2.8

dihedrals defined by three last C−C bonds of the γ-chains. As the temperature was increased from 308 to 363 K, the proportion of *gauche* defects grew by about 12%.

The basic simulation results are summarized in Table I.[25] At both separations tried, the increase in temperature led to slight, albeit statistically significant, dehydration of the membranes. The decrease in n_w on heating from 308 to 363 K was about 7% and 5% at $h=38.6$ and 39.6 Å, respectively. Note that the decrease in the bulk water density in the same temperature range is only 3%.

The effect of temperature on the intermembrane pressure p and its components at $h=39.6$ Å is comparable with the probable error (0.2 kbar), so we can only note a likely trend of p to decrease with temperature. At the shorter separation, $h=38.6$ Å, the effect of temperature is more definite. As the temperature is increased from 308 to 363 K, the total pressure p falls by about a quarter of magnitude, mainly due to a decrease in p_h.

To conclude, the above-described simulation results are in qualitative agreement with the experimental measurements by Simon *et al.*[8] in that the short-range repulsion between fluid-phase phospholipid membranes tends to decrease with increasing temperature. Note that the comparison of our simulation results for supported membranes with the osmotic stress data for multi-bilayer lipid suspensions[8] is justified by the fact that at pressures of the order of 1 kbar, as considered in our simulations, the separation between fluid-phase bilayers in the osmotic stress measurements was only $2-3$ Å and so the undulation pressure, which was neglected in our simulations, was insignificant.

The observed decrease in p with temperature seems to originate, at least in part, from temperature-induced dehydration of the membranes and the associated decrease in p_h. It should be reminded that our simulations were performed at a fixed areal density of the membranes, unlike the experimental measurements[8] where the membranes were free to change their areal density depending on pressure and temperature. It can, however, be anticipated that the short-range repulsion between the simulated membranes would show an even stronger decrease with temperature if the membranes would be allowed to expand. The reason is that the lateral expansion of the membranes should decrease the areal density of their hydrophilic headgroups and make the region of the hydrophobic tails more accessible to water. Both of these factors should diminish the overall hydrophilicity of the membranes, with the result that the hydration pressure should decrease.

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[21] Although modern MD simulations of phospholipid membranes are performed, for the most part, with 64 lipids per monolayer, the simulation boxes containing $32-36$ lipid molecules have been shown (Ref. 22) to be large enough to reproduce the main structural and dynamical features of hydrated lipid bilayers. The obvious inability of the so small systems to reproduce membrane undulations is of no concern of simulations of supported membranes, where the undulations are suppressed anyhow.

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[25] Compared to our previous results at $T=308$ K (Ref. 11), the present values of n_w are somewhat higher, whereas those of p are lower. The reasons are the use of substantially longer GCMC runs, the averaging of the simulation results over a series of independent runs, and the use of the rotational bias procedure, which noticeably enhanced the sampling efficiency. All these factors promoted better equilibration of the system and made the sampling more representative.